



OCCASIONAL PAPERS

Museum of Texas Tech University

Number 391

28 January 2025

MORPHOLOGICAL MEASUREMENTS ARE INSUFFICIENT TO DIFFERENTIATE *MUSTELA RICHARDSONII* AND *NEOGALE FRENATA*: IMPLICATIONS FOR DOCUMENTING POSSIBLE RANGE EXPANSION

JAMES J. KRUPA AND KATHRYN M. EVERSON

ABSTRACT

In 2019, an adult female weasel was found in Henry County, Kentucky, near the Indiana border. Identification as *N. frenata* could not be assumed based on external morphology alone, as the specimen closely resembled *Mustela richardsonii*. Although *Mustela richardsonii* is not known from Kentucky, it has been reported from central Indiana and central Ohio. Twenty-one morphometric measurements were recorded from the unidentified weasel. Eight were in the range for *M. richardsonii*, two in the range for *N. frenata*, and 11 were intermediate between the two species. Consequently, genetic analysis was performed. From this, the specimen was confidently assigned to *N. frenata* based on an average of 99.0% sequence identity to other *N. frenata* specimens. Similar to the recent southern expansion of the Least Weasel (*M. nivalis*) into Kentucky and Tennessee, it is possible *M. richardsonii* could be an occasional transient in Kentucky. Thus, morphologically ambiguous weasels with short, black-tipped tails encountered in Kentucky may require morphometric and genetic analysis to definitively identify the species.

Key words: genetic analysis, geographic distribution, Kentucky, morphometrics, *Mustela richardsonii*, *Neogale frenata*

INTRODUCTION

American Ermine (*Mustela richardsonii*) and Long-tailed Weasels (*Neogale frenata*) have extensive overlapping ranges in North America (Hall 1951; Simms 1979; Hall 1981; Fagerstone 1987). In eastern North America, *M. richardsonii* occurs from the Canadian Arctic south to Pennsylvania, Ohio, Indiana, northern Iowa, and South Dakota (Fagerstone 1987; Cheeseman et al. 2024; Fig. 1). In eastern North America, *Neogale frenata* occurs in southern Canada

southward to the Gulf of Mexico (Patterson et al. 2021; Fig. 1). Confirming the species of individuals in areas of sympatry can be challenging as both species show geographic variation in size and have overlapping morphological measurements (St-Pierre et al. 2006). This challenge is compounded because both species exhibit extreme sexual dimorphism, with females being smaller than males. Thus, species identification based on external morphology alone could result in errors.

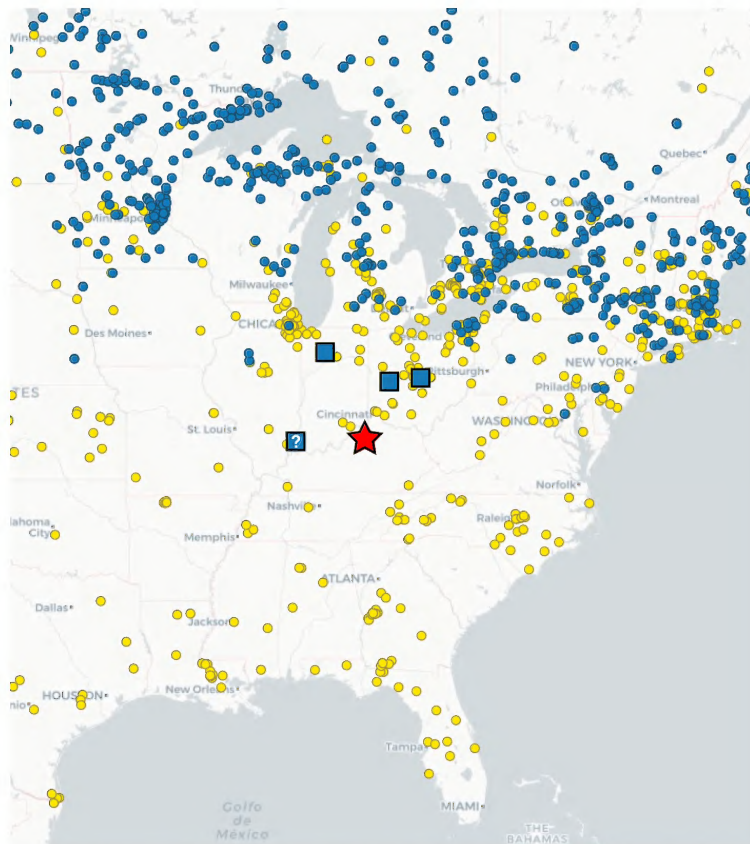


Figure 1. Map showing locality records for *Mustela richardsonii* (blue dots; GBIF data: 10.15468/dl.59p64j) and *Neogale frenata* (yellow dots; GBIF data: 10.15468/dl.t8mzvz). Blue squares represent specimens identified as *M. richardsonii* from Lafayette, Indiana, Urbana, Ohio, and West Berlin, Ohio. The blue square with question mark is for an iNaturalist photo of questionable species identification from Vienna, Illinois. The red star represents the Henry County specimen examined in this study. Map was generated in QGIS using basemap tiles from CartoDB (<https://cartodb.com/basemaps/>).

Efforts have been made to establish criteria for identifying weasel species using external morphological traits. Hall (1951) stated that *M. richardsonii* has a tail length to head and body length ratio ≤ 0.45 and *N. frenata* has a ratio > 0.45 . Others have stated that a ratio of < 0.44 is considered a trait for *M. richardsonii* (King 1983), whereas a ratio of > 0.44 is considered a trait for *N. frenata* (Sheffield and Thomas 1997). By

using genetic analysis, St-Pierre et al. (2006) found that species identification based on morphology alone was incorrect for 18% of specimens examined from the St. Lawrence River Valley in eastern Quebec, Canada. These results suggest the accepted ranges for both species may not be accurate when relying only on morphological traits to determine species (St-Pierre et al. 2006). These authors suggested the zone of sym-

patry for the two weasels may be wider than currently thought because *N. frenata* may have a range farther north than currently accepted (St-Pierre et al. 2006). This could be a growing issue if *M. richardsonii* expands its range southward.

There are no confirmed records, and no suggestion has been made, that *M. richardsonii* occurs in Kentucky. However, it is not beyond the realm of possibility that this species might expand its range into Kentucky, as with other weasel species. The Least Weasel (*M. nivalis*), for example, originally was not known to be in Kentucky (Barbour and Davis 1974) until 1976 (Davis and Barbour 1979), and since then, this species has been reported from more than 15 counties in the state (Meade 1992; Krupa unpub. data). Recent specimens from Tennessee provide further evidence that Least Weasels are continuing to expand their range southward (Barnes and Hoffman 2023). Thus, it cannot be assumed that *M. richardsonii* is incapable of expanding southward as well. Based on this, small weasels assumed to be *N. frenata* need to be examined carefully when south of the accepted range for *M. richardsonii*.

An adult female weasel was found dead on 3 January 2019 on Hieatt Road approximately 8 km west of New Castle, Henry County, Kentucky (GPS coordinates: 38.41783, -85.24050; Figs. 1 and 2). Henry County is along the Ohio River adjacent to Indiana. It appears this individual was killed by another weasel based on 15 small puncture wounds over the ventral rib cage, shoulders, neck, and cranium, plus there was extensive hemorrhaging around the neck and head with damage to the cranium (Fig. 2c). This was a small individual with a short tail. The tail was complete based on presence of the distal-most caudal vertebra, and the black fur on the tip of the tail was complete (Fig. 2c).

The ratio of tail length divided by combined head and body length was 0.44.

GBIF records for *M. richardsonii* (10.15468/dl.59p64j) indicate three possible specimens relatively close to Kentucky (Fig. 1). A female *M. richardsonii* was collected in 1962 from Lafayette, Indiana (specimen deposited at Western New Mexico University, WNMU-1229), 310 km north of New Castle, Kentucky. In 1948, a male *M. richardsonii* was collected from Urbana, Ohio (specimen deposited in the University of Kansas Biodiversity Institute, KUBI-154210), 267 km northeast of New Castle. On 23 September 1988, a female was collected near Berlin, Ohio (specimen deposited in the Ohio State University Museum, OSUM Mammals 3931), 327 km northeast of New Castle. We have not examined these specimens; but if identification is correct, they are south of the accepted range for this species (Fagerstone 1987; Cheeseman et al. 2024; Fig. 1). In addition, a photograph was taken of a roadkill weasel near Vienna, Illinois, 20 km north of Kentucky (<https://www.inaturalist.org/observations/50348880>) resembling *M. richardsonii*. There is disagreement over which species this represents, with one opinion being it is too far south to be *M. richardsonii*. These records suggest it is possible, however, that *M. richardsonii* could occur farther south than commonly accepted, but that it remains undocumented due to misidentification.

After careful examination of external morphology, the species of the Henry County specimen could not be confirmed. Although it was assumed to be *N. frenata* based on the lack of records of *M. richardsonii* in Kentucky, the authors deemed it appropriate to conduct morphometric and genetic analyses to confirm this identification.

METHODS

Morphometrics.—A 500 g Pesola balance was used to measure body mass to the nearest gram. A Mettler balance was used to measure the mass of cranium and lower jaw to the nearest 0.1 gram. Total length, tail length, hind foot and ear length were measured to the nearest millimeter. Total length and tail length were measured to the fleshy tip of the tail. The number of caudal vertebrae was counted, and the

skull measurements (see Fig. 3) were taken following Elsasser and Parker (2008). These include: condylo-basal length (LCB), basilar length (LB), postglenoidal length (LPG), mastoid width (Ma), cranial width (Bc), bi-zygomatic width (Bz), muzzle width (Ro), foramen magnum diameter height and width, palatine to rostrum length, palatine to pterygoid process length, and mandibular angular to coronoid process height (ACPH).

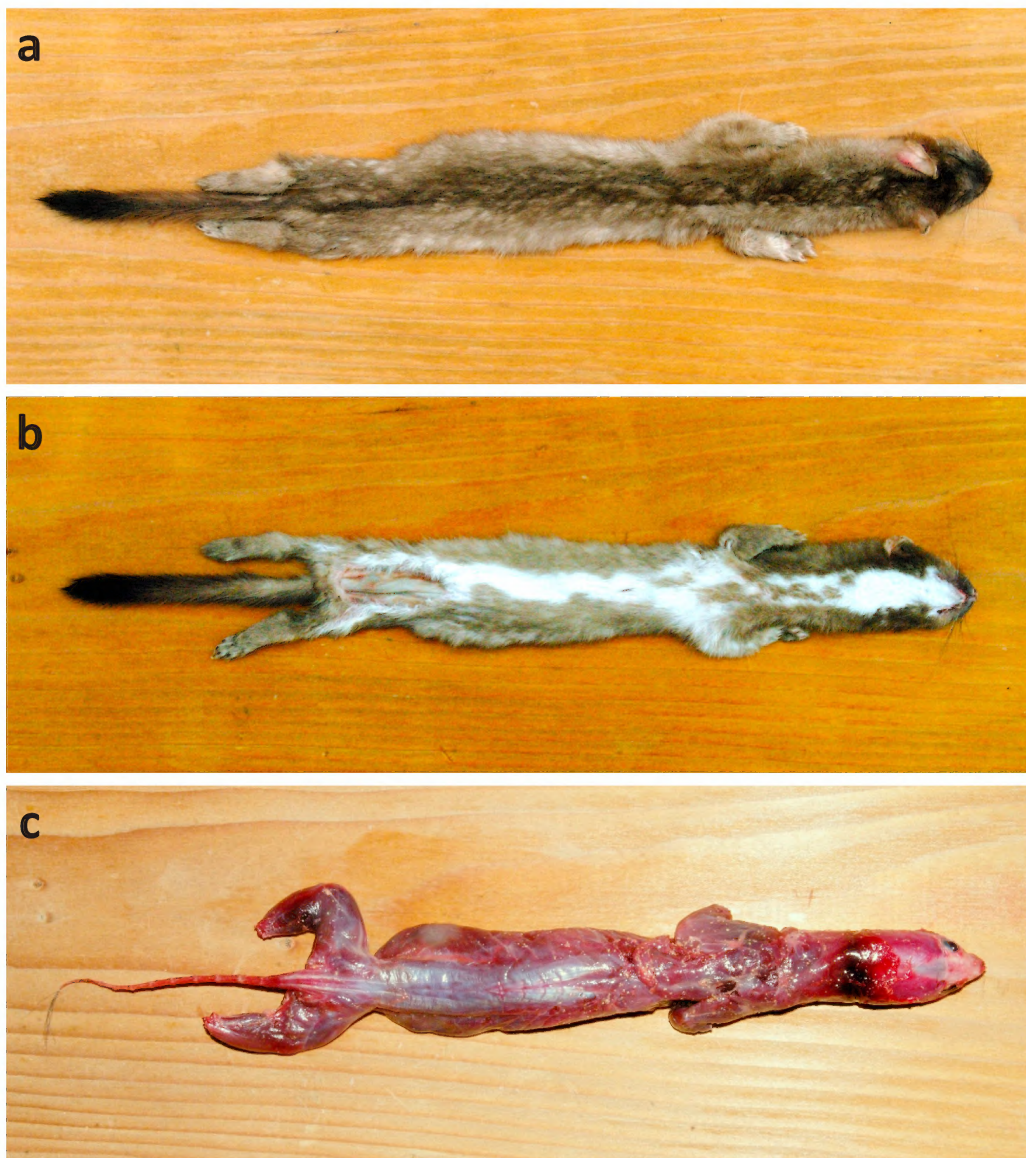


Figure 2. a) Photograph of dorsal side of the Henry County, Kentucky weasel after being skinned for preparation as a voucher specimen; b) photograph of ventral side after being skinned; c) photograph of the carcass. Note the strands of black fur on tail attached to the distal-most caudal vertebra. Tooth marks likely from another weasel can be seen on the shoulder. Hemorrhaging on neck and base of skull is from bite wounds.

Measurements were taken with digital calipers to 0.01 mm (Fig. 3).

The specimen was prepared as a scientific voucher skin and skeleton and archived in the Mammalogy Collection of the University of Kansas Biodiversity Institute and Natural History Museum (KU 173850).

Genetic data collection and analysis.—Samples of heart, striated muscle, and liver tissue were preserved in 100% ETOH for genetic analysis. Genomic DNA was extracted from muscle tissue using a DNeasy blood and tissue kit (Qiagen). PCR amplification was performed for the mitochondrial cytochrome-*b* gene

(*cyt-b*) using previously published primers (St-Pierre et al. 2006): MUSCYBF (5'-QTG ACC AAC ATT CGT AAA-3') and MUSCYBR (5'-CAA AAT GAT ATT TGT CCT-3'). PCR parameters were as follows: an initial stage of three minutes at 94°C; 35 cycles followed the initial cycle in the following order: a denaturation stage of 30 seconds at 94°C, an annealing stage of 30 seconds at 50°C, and an extension stage of 1 minute at 72°C; after 35 cycles, there was a final extension stage of 4 minutes at 72°C and the mixture was held at a constant 4°C. Raw PCR products were purified and sequenced on a Sanger instrument by Psomagen Inc. (Rockville, Maryland, USA).

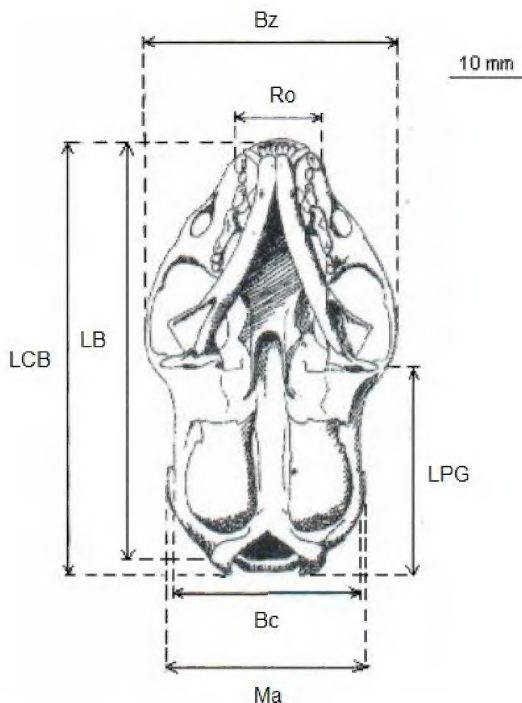


Figure 3. Drawing of weasel skull, mandible, and measurements from Elsasser and Parker (2008), which was adapted from Debrot and Mermod (1978). The measurements are as follows: condylobasal length (LCB), basilar length (LB), postglenoidal length (LPG), mastoid width (Ma), cranial width (Bc), bi-zygomatic width (Bz), muzzle width (Ro).

Raw chromatograms were trimmed and edited by eye, then forward and reverse strands were assembled using Geneious Prime version 2020.0.5 (Biomatters Ltd. 2020). To determine the species identity, Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov>) was used to compare our DNA sequence to *cyt-b* sequences of known species identity available

on the National Center for Biotechnology Information (NCBI) GenBank nucleotide database (Benson et al. 2013). Taxonomic assignment was determined using the criterion of sequence identity, which measures the percent of our sequence that is identical to other sequences of known species assignment.

RESULTS

Examination of the skeleton revealed this was an adult weasel based on the lack of epiphyseal growth plates and the permanent and full emergence of all teeth. This weasel had a total length of 300 mm, tail length of 92 mm, hind foot length of 35 mm, ear length of 20 mm, and a mass of 102 g. Of the 21 morphometric measurements recorded (Table 1), only two of the measurements (body mass and skull mass) were in the range of female *N. frenata* provided by Elsasser and Parker (2008). Seven of the measurements were in the range of female *M. richardsonii* (postglenoidal length, mastoid width, cranial width, foramen magnum width, muzzle width, foramen magnum height, and palatine to pterygoid process length). In addition, the number of tail vertebrae was lower than for *M. richardsonii*

despite no missing caudal vertebrae. Thus, this can be scored with the other *M. richardsonii* measurements. Eleven of the measurements were less than the lowest value for female *N. frenata*, but greater than the highest values provided for female *M. richardsonii* (body length, head and body length, tail length, tail to head and body length ratio, mandible mass, condylo-basal length, basilar length, postglenoidal-condylobasal length ratio, by-zygomatic width, palatine to rostrum length, and angular to coronoid process height) based on Elsasser and Parker (2008).

After trimming and assembling the raw sequence data, a 423 base-pair (bp) length fragment of the mitochondrial *cyt-b* gene was obtained (GenBank accession

Table 1. Twenty-one morphometric measurements of the adult female weasel from Henry County, Kentucky compared to morphometric measurements of females taken by Elsasser and Parker (2008). Green font indicates a measurement within the range of *Neogale frenata*. Blue font indicates a measurement within range of *Mustela richardsonii*. Red font indicates measurements smaller than for *N. frenata* and larger than for *M. richardsonii*, or within the range of both. Ranges of measurements are in parentheses.

	Juvenile <i>M. richardsonii</i> (n = 3)	Adult <i>M. richardsonii</i> (n = 2)	Henry County Weasel	Juvenile <i>N. frenata</i> (n = 1)	Adult <i>N. frenata</i> (n = 3)
Body mass (g)	55.02 (40.46–79.06)	43.38 (38.71–48.05)	102	105.91 (88.1–135.5)	98.7 (80.7–114.0)
Body length (mm)	253 (232–280)	240 (233–248)	300	333 (315–362)	329 (316–350)
Head and body length (mm)	184 (171–196)	179 (174–184)	208	218 (210–228)	219 (210–227)
Tail length (mm)	69 (61–84)	62 (59–64)	92	115 (105–134)	110 (104–123)
Tail to head and body length ratio	0.38 (0.34–0.43)	0.35 (0.34–0.35)	0.44	0.53 (49–59)	0.50 (48–54)

Table 1. (cont.)

	Juvenile <i>M. richardsonii</i> (n = 3)	Adult <i>M. richardsonii</i> (n = 2)	Henry County Weasel	Juvenile <i>N. frenata</i> (n = 1)	Adult <i>N. frenata</i> (n = 3)
Number of caudal vertebrae	17.5 (17–18)	17.5 (17–18)	14	19.0 (19–20)	20 (19–21)
Skull mass (g)	0.66 (0.50–0.83)	-	1.30	1.43 (1.22–1.63)	1.33 (1.17–1.45)
Mandible mass (g)	0.21 (0.17–0.26)	-	0.35	0.48 (0.41–0.54)	0.43 (0.38–0.46)
Condylobasal length (mm)	36.28 (34.17–38.51)	-	38.56	41.8 (40.40–43.10)	41.81 (40.47–42.94)
Basilar length (mm)	33.80 (31.99–36.0)	-	36.78	38.67 (36.86–40.47)	38.22 (37.17–39.52)
Postglenoidal length (mm)	17.98 (16.81–19.08)	-	18.85	20.48 (19.71–21.25)	20.69 (20.31–21.05)
Postglenoidal-condylo- basal length ratio	0.50 (0.44–0.53)	-	0.49	0.49 (-)	0.50 (0.48–0.50)
Mastoid width (mm)	16.59 (15.40–18.11)	-	18.05	19.41 (18.66–20.16)	18.98 (18.41–19.90)
Cranial width (mm)	16.62 (15.30–17.73)	-	17.54	18.85 (18.36–19.39)	18.52 (18.24–18.81)
Bi-zygomatic width (mm)	18.11 (16.58–19.46)	-	19.52	21.12 (21.53–22.70)	21.25 (20.65–21.57)
Muzzle width (mm)	8.64 (7.95–9.13)	-	7.88	9.53 (8.46–10.59)	9.41 (9.06–9.79)
Foramen magnum height (mm)	5.40 (5.32–5.52)	-	5.04	5.38 (4.92–5.84)	5.56 (4.66–5.88)
Foramen magnum width (mm)	6.41 (6.25–6.67)	-	6.20	7.03 (6.21–7.84)	6.98 (6.31–7.30)
Palatine to rostrum length (mm)	14.27 (13.28–15.69)	-	15.75	16.97 (16.83–17.11)	17.38 (16.68–17.80)
Palatine to pterygoid process length (mm)	5.97 (5.88–6.17)	-	5.32	6.81 (6.35–7.26)	6.57 (5.90–7.12)
Angular to coronoid process height (mm)	8.13 (7.53–8.88)	-	9.51	10.45 (9.71–11.19)	10.14 (9.63–10.48)

number - OR888449). Comparison of this sequence against the NCBI's GenBank nucleotide database using BLAST resulted in 132 hits to North American mustelids (Table 2). From this, the Henry County

weasel is confidently assigned to *N. frenata* based on an average of 99.0% sequence identity to other *N. frenata* specimens in GenBank (compared to ~91% sequence identity to other species).

Table 2. Results of the BLAST comparison of *cyt-b* sequences to data in GenBank to assist in species identification.

Species	Number of Hits	Avg. % Identity (Range)
<i>Neogale frenata</i>	72	99.0% (98.6–99.7%)
<i>Mustela richardsonii</i>	45	91.3% (91.3–91.5%)
<i>Neogale vison</i>	15	91.6% (91.5–92.0%)

DISCUSSION

Comparing the morphometric measurements for the Henry County weasel to those provided by Elsasser and Parker (2008) revealed ambiguous results for some of the measurements, suggesting the weasel could be either *M. richardsonii* or *N. frenata*. Thus, morphological traits alone could not determine its species. Consequently, genetic analysis was essential for identification. This analysis confirmed the Henry County weasel as *N. frenata*.

Females of both *M. richardsonii* and *N. frenata* are rarely trapped (St-Pierre et al. 2006; Elsasser and Parker 2008). As a result, St-Pierre et al. (2006) had too few females for genetic analysis, while Elsasser and Parker (2008) had small sample sizes of females for both species. This included no skulls of adult female *M. richardsonii*. If larger sample sizes were available, more (if not most) of the ambiguous measurements of the Henry County female may have fallen within the ranges of one of the two species. Because of overlaps

in the range of morphological measurements, relying on these alone will cause occasional misidentification. With *M. richardsonii* thought to have a more limited range than *N. frenata*, range expansion of *M. richardsonii* could go undetected when individuals are similar in size to *N. frenata*. Thus, a genetic analysis should be performed on those individuals that are morphologically ambiguous.

Genetic analyses are not known to have been done on the three specimens from Indiana and Ohio to confirm species. However, if correctly identified, this indicates *M. richardsonii* has been found a relatively short distance from northern Kentucky. This suggests it is possible for transient individuals to occur even farther south from their accepted range. Thus, small weasels with short, black-tipped tails must have their morphometrics examined carefully along with genetic analysis in the event *M. richardsonii* does occasionally occur farther south than assumed.

ACKNOWLEDGMENTS

We thank Jonathan A. Matthews for finding and donating the specimen. We also thank the editors of

Acta Zoologica Academiae Scientiarum Hungaricae for allowing us to use the drawing in Figure 3.

LITERATURE CITED

- Barbour, R. W., and W. H. Davis. 1974. Mammals of Kentucky. University Press of Kentucky, Lexington.
- Barnes, E. F., and J. D. Hoffman. 2023. Significant range expansions in eight species of North American mammals. Occasional Papers, Museum of Texas Tech University 385:1–29.
- Benson, D. A., M. Cavanaugh, K. Clark, I. Karsch-Mizrachi, D. J. Lipman, J. Ostell, and E. W. Sayers. 2013. GenBank. Nucleic Acids Research, 41(D1), pp. D36–D42.
- Cheeseman, A. E., D. S. Jachowski, and R. Kays. 2024. From past habitats to present threats: tracing North American weasel distributions through a century of climate and land use change. Landscape Ecology 39:article 104.
- Davis, W. H., and R. W. Barbour. 1979. Distributional records of some Kentucky mammals. Transactions of the Kentucky Academy of Science 40:111.
- Debrot, S., and C. Mermoud. 1978. Morphométrie crânienne par radiographie. Revue Suisse de Zoologie 85:730–738.
- Elsasser, S. C., and G. H. Parker. 2008. Morphometric criteria for distinguishing species and age cohorts of ermine (*Mustela erminea*) and long-tailed weasel (*M. frenata*). Acta Zoologica Academiae Scientiarum Hungaricae 54:75–88.
- Fagerstone, K. 1987. Black-footed ferret, long-tailed weasel, short-tailed weasel, and least weasel. Pp. 555–561 in Wild furbearer management and conservation in North America (M. Novak, J. A. Baker, M. E. Fobbard, and B. Malloch, eds.). Ontario Ministry of Natural Resources, Ontario, Canada.
- Hall, E. R. 1951. American weasels. University of Kansas Museum of Natural History Publications 4:1–466.
- Hall, E. R. 1981. The mammals of North America, 2nd ed. 2 vols. John Wiley & Sons, New York.
- King, C. M. 1983. *Mustela erminea*. Mammalian Species 195:1–8.
- Meade, L. 1992. New distributional records for selected species of Kentucky mammals. Transactions of the Kentucky Academy of Science 53:127–132.
- Patterson, B. D., H. E. Ramírez Chaves, J. F. Vilela, A. E. R. Soares, and F. Grewe. 2021. On the nomenclature of the American clade of weasels (Carnivora: Mustelidae). Journal of Animal Diversity 3:1–8.
- Sheffield, S. R., and H. H. Thomas. 1997. *Mustela frenata*. Mammalian Species 570:1–9.
- St-Pierre, C., J.-P. Ouellet, F. Dufresne, A. Chaput-Barday, and F. Hubert. 2006. Morphological and molecular discrimination of *Mustela erminea* (Ermines) and *M. frenata* (Long-tailed weasels) in eastern Canada. Northeastern Naturalist 13:143–152.

Addresses of authors:

JAMES J. KRUPA

Department of Biology
University of Kentucky
Lexington, KY 40503
bio149@uky.edu

KATHRYN M. EVERSON

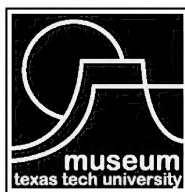
Department of Integrative Biology
Oregon State University
Corvallis, OR 97333
eversonk@oregonstate.edu

PUBLICATIONS OF THE MUSEUM OF TEXAS TECH UNIVERSITY

This publication is available free of charge in PDF format from the website of the Natural Science Research Laboratory, Museum of Texas Tech University (www.depts.ttu.edu/nsrl). The authors and the Museum of Texas Tech University hereby grant permission to interested parties to download or print this publication for personal or educational (not for profit) use. Re-publication of any part of this paper in other works is not permitted without prior written permission of the Museum of Texas Tech University.

Institutional subscriptions to Occasional Papers are available through the Museum of Texas Tech University, attn: NSRL Publications Secretary, Box 43191, Lubbock, TX 79409-3191. Individuals may also purchase separate numbers of the Occasional Papers directly from the Museum of Texas Tech University.

Series Editor: Robert D. Bradley
Production Editor: Lisa Bradley
Copyright: Museum of Texas Tech University



ISSN 0149-175X

Museum of Texas Tech University, Lubbock, TX 79409-3191